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Seroepidemiological survey of *Brucella canis* infection in dogs in Japan

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Abstract

A seroepidemiological survey of *Brucella canis* infection was performed on 1,071 dogs (companion, stray, and shelter groups) from seven prefectures in Japan. Overall, 3.5% (38/1,071) of the dogs were seropositive for *B. canis* by microplate agglutination test although no clinical signs were observed in positive animals. The seroprevalence by dog group was 2.9% (24/835) in the companion group, 3.6% (4/110) in the stray group, and 7.9% (10/126) in the shelter group. A significant difference was found between shelter and companion groups ($P < 0.01$). Our study shows that asymptomatic infection of *B. canis* is widely distributed among dogs in Japan, and rearing in a group under poor hygienic conditions may lead to a risk of exposure to *B. canis*.

Keyword: Animal shelter; *Brucella canis*; Dog.

Canine brucellosis is a zoonosis caused by *Brucella canis*. Though most dogs infected with *B. canis* are asymptomatic, some dogs show reproductive disorders such as abortion, testitis, and epididymitis. On the other hand, major symptoms in humans are fever and arthritis. *B. canis* can be transmitted to dogs and humans by the contact of genitourinary and vaginal secretions and/or by direct contact with infected dogs.

Recently, canine brucellosis has drawn attention as a public health risk because the number of pet dogs is increasing in developed countries and owners are in close contact with dogs at home[4)]. Therefore, dogs infected with *B. canis* rearing under low hygienic conditions may be a risk factor for human brucellosis. Although human brucellosis from dogs is rare in Japan[7)], a case was reported in 2008 in pet shop owners who handled aborted placentas of infected dogs, and *B. canis* was isolated from both the patients and five breeding dogs[11)]. Furthermore, a veterinarian was infected with *Brucella* sp. and showed fever and numbness of the hands in 2018. Even though the source of infection was not confirmed, there was a case that the patient was suspected to be affected by the disease from dogs[2)].

In Japan, it was reported that 3.0 to 8.3% of companion dogs were seropositive for *B. canis* in 2011 and 2014[10, 12)]. However, comparison of the infectious status in stray, shelter, and companion dogs has not been available so far. The present study was conducted to clarify the present status of *B. canis* infection in stray, shelter,

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and companion dogs in Japan, and to determine potential risk factors of canine brucellosis transmission to humans by different dog groups.

A total of 1,071 (571 male and 500 female) serum samples were collected from dogs in 7 prefectures including Kanagawa, Chiba, Fukushima, Aichi, Fukuoka, Tottori, and Tokushima Prefectures in Japan between 2009 and 2017. Frozen serum samples were sent to the Laboratory of Veterinary Public Health, Nihon University and kept at -20 °C until examination. Dogs were categorized into three groups: companion (n = 835), stray (n = 110), and shelter (n = 126). The companion group were dogs reared as household pets in 6 prefectures (Kanagawa, Chiba, Aichi, Fukuoka, Tottori, and Tokushima) and informed consent was obtained from all dog owners before collecting serum samples. The stray group included free-ranging dogs captured in the field of Tokushima Prefecture. The shelter group consisted of former companion dogs that left their homes in Fukushima Prefecture for several weeks to several months after the Great East Japan Earthquake of 2011. These dogs were captured and sent to one dog shelter in Kanagawa Prefecture, and then kept in a group segregated by sex for 2 to 3 months.

Specific antibodies to *B. canis* were identified by a microplate agglutination test as previously described[9]. Briefly, 25 µl of serum were added to the well of U-bottom of 96 well microplate (Stem Corporation, Tokyo, Japan) and two-fold serial dilutions were made by phosphate buffered saline. Then, an equal volume of *B. canis* QE13B strain antigen solution (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) containing 0.005% safranin solution (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) was added to each well. A rabbit serum immunized with *B. canis* antigen was used as a positive control. The plates were sealed, mixed gently for 20 seconds, and incubated at 50°C for 24 hours. An agglutination titer greater than 1:160 was defined as positive. Seroprevalence was compared by sex and dog groups (companion, stray, and shelter groups) using chi-square test with P values < 0.01 considered statistically significant.

Overall, *B. canis* antibody was detected in 3.5% (38/1,071) of the dogs examined, but none of the dogs showed any clinical signs of brucellosis. Tachibana et al.[12] also showed similar seroprevalence (3.0%) of *B. canis* infection in companion dogs in Japan. The seroprevalence was 3.5% (20/571) in males and 3.6% (18/500) in females and no significant difference was observed between both sexes (Table 1). In previous studies, no significant difference in the seroprevalence was also observed by sex in Japan[9, 10, 12], China[13], and Zimbabwe[3]. Thus, there seems to be no difference in the opportunity to exposure to *B. canis* in dogs by sex.

Antibodies to *B. canis* were detected in 2.9% (24/835) of the companion group, 3.6% (4/110) of the stray group, and 7.9% (10/126) of the shelter group (Table 1). In the present study, the seroprevalence in companion group was low and approximately the same as a previous study (3.0%) in Japan[12]. About half of companion dogs in Japan are estimated to be neutered and breeding is strictly controlled by the owners[8]. As a result, we speculate that the companion group had fewer opportunities to be exposed to *B. canis* by breading. In the United States, the higher seroprevalences of *B. canis* were found in stray and free-roaming dogs in the rural areas of the southeast[1,5]. Although stray dogs were also thought to have more opportunities to acquire *B. canis* infection than companion dogs in Japan, no significant difference was found in the seroprevalence between both the groups. In contrast, the seroprevalence in the shelter group was significantly higher than that in the companion group (P < 0.01). Dogs in the shelter group had been roaming freely in the Fukushima Prefecture for several weeks to several months after the Great East Japan Earthquake in 2011. Then, these dogs were captured and admitted to an animal shelter in Kanagawa Prefecture for several months. It is reported that 4.9% of shelter dogs were seropositive for *B. canis* in Mississippi,

Table 1. Seroprevalences of *B. canis* by sex in three dog groups.

Sex	Companion group		Stray group		Shelter group		Total	
	No. tested	No. positive (%)	No. tested	No. positive (%)	No. tested	No. positive (%)	No. tested	No. positive (%)
Male	435	11 (2.5)	61	2 (3.3)	75	7 (9.3)	571	20 (3.5)
Female	400	13 (3.6)	49	2 (4.1)	51	3 (5.9)	500	18 (3.6)
Total	835	24 (2.9)*	110	4 (3.6)	126	10 (7.9)*	1071	38 (3.5)

*: Values significantly different ($P < 0.01$) between groups are labelled with an asterisk.

Table 2. Seroprevalence of *B. canis* in companion dogs compared by prefecture

Prefecture	No. tested	No. positive (%)
Kanagawa	297	9 (3.0)
Chiba	264	4 (1.5)
Aichi	96	1 (1.0)
Fukuoka	85	7 (8.2)
Tottori	54	0
Tokushima	39	3 (7.7)
Total	835	24 (2.9)

USA, and mean seroprevalence was 17.8% for the shelters where *B. canis* infection[6] was present. Though we could not determine whether shelter dogs were exposed to *B. canis* prior to capture or during stay in the shelter, our results indicate that tight spaces such as a shelter and rearing dogs in a group for a long period of time are responsible for the high seroprevalence of *B. canis*. Spay and neuter of dogs prior to adoption and public education including understanding the clinical signs of the disease and good hygienic practices may effectively reduce the opportunities of *B. canis* infection to dogs and humans.

The seroplevalence of companion dogs by prefecture varied from 1.0% in Aichi Prefecture to 8.2% in Fukuoka Prefecture. No positive dogs were detected in Tottori Prefecture (Table 2). Hubbard *et al*. also showed seroprevalence by district ranged from 0% to 6.3% in Mississippi, USA[6]. Thus, the seroprevalence of canine brucellosis in companion dogs differed by the prefecture and/or district examined.

Our results revealed that asymptomatic *B. canis* infection is still widely distributed among dogs in Japan, although at a low rate, and that seroprevalence is associated to the living or rearing conditions of dogs. Dogs especially reared in a group under poor hygienic conditions have a significant risk to be exposed to *B. canis*. Continuous seroepidemiological surveys are necessary to control the risk of *B. canis* infection for both humans and dogs.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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